

## Karyomorphological Analysis of Diploid Races of *Aucuba japonica* Thunb. (Cornaceae)

Machiko TSUSAKA, Hiroshi IKEDA and Takuji HOSHINO

Department of Mathematical and Environmental System Science, Graduate School of Informatics,  
Okayama University of Science, 1–1, Ridai-cho, Okayama 700-0005 JAPAN  
E-mail: ikeda@big.ous.ac.jp

(Received on October 6, 2005)

A karyomorphological analysis of diploid races of *Aucuba japonica* Thunb., with special reference to their sexualities, was carried out. After examining 59 individuals from 16 localities in the Chugoku and Shikoku Districts, western Japan, all individuals proved to be diploid with  $2n = 16$ , and the smallest pair of chromosomes had a satellite on each short arm. Among the 59 individuals, 24 individuals (“heterotype”) possessed a heterogeneous pair of chromosomes composed of median and submedian chromosomes in the fourth longest pair. The remaining 35 individuals (“homotype”) possessed homogeneous pair with median chromosomes in the fourth longest pair. Such cytological variation did not show a geographical tendency, but all the “heterotypes” were observed in male individuals, and “homotypes” were in female individuals. Therefore we have concluded that the karyomorphological variations in diploid races of *A. japonica* depend on their sexualities.

**Key words:** *Aucuba japonica*, diploid race, karyomorphology, sex chromosome.

*Aucuba japonica* Thunb. (Cornaceae) is distributed throughout Japan, from southern Hokkaido to Okinawa, and in Korea and Taiwan. It is an evergreen dioecious shrub mainly growing in temperate forests. *Aucuba japonica* is usually classified into three varieties, vars. *japonica*, *borealis* Miyabe & Kudo, and *ovoidea* Koidz. Variety *japonica* is distributed from the Tohoku District to the Chugoku and Shikoku Districts mainly on the Pacific Ocean side. Variety *borealis* is distributed from southern Hokkaido to the Kinki District through the Tohoku and Hokuriku Districts, mainly on the Japan Sea side. Variety *ovoidea* is distributed from eastern part of the Chugoku and Shikoku Districts to the Kyushu District and also in the Amami and Okinawa Islands (Hara 1966, 1989, Noshiro 1999, Ohi et al. 2003).

Chromosome numbers have been reported to be  $2n = 32$  (tetraploid) for vars. *japonica* and *borealis* and to be  $2n = 16$  (diploid) for var. *ovoidea* (Meurman 1929, Funabiki 1958, Kurosawa 1971). Karyotype analyses have been made mainly for tetraploid races (Meurman 1929, Sinoto 1929, Viinikka 1970), and such studies of diploid races have not been extensively carried out. Kurosawa (1971) compared the karyotypes of diploid and tetraploid races.

Sex chromosomes have been reported in several dioecious plants, mainly in herbs such as *Rumex* (Kihara and Ono 1923, Löve 1943), *Melandrium* (Blackburn 1923, Winge 1923), *Humulus* (Winge 1923), and *Cannabis* (Sinoto 1929), and relatively few in woody plants such as *Salix* (Blackburn and Harrison 1924), and *Palmae* (Sinoto

1929). On the genus *Aucuba*, no sex chromosome has been reported. Sugiura (1927), Meurman (1929) and Sinoto (1929) observed chromosomes of tetraploid races of *A. japonica* and noted that there was no sex chromosome among 32 somatic chromosomes. Kihara and Yamamoto (1935) examined *A. chinensis* ( $2n = 16$ ), and Kurosawa (1971) examined *A. chinensis* and *A. himalaica* ( $2n = 16$ ) cytologically, but they did not mention sex chromosomes.

This paper aims to provide an accurate report of karyomorphology of diploid races of *A. japonica*, with special reference to sex or sex-dependent chromosomes.

### Materials and Methods

Fifty-nine individuals were collected from 16 localities in the Chugoku and Shikoku Districts, western Japan (Table 1). Young branches were propagated hydroponically and were also arranged as herbarium specimens. Root tips were pretreated with 0.002 M 8-hydroxyquinoline solution for an hour at 20°C and stored 15 hours at 4°C, then they were fixed with a 3:1 mixture of 99.5 % ethanol and glacial acetic acid at -20°C more than one hour. The root tips were hydrated and were soaked in a 1N HCl solution for 10 minutes at 60°C, and were transferred to Schiff's reagent at room temperature for an hour. Then the materials were macerated with a solution of a mixture of 2 % cellulase and 2 % pectinase for 30 minutes at 37°C, and washed in distilled water. The meristematic tissues were placed on slide glasses and were squashed with an acetoglycerin (45 % acetic acid with a small amount of glycerin) and observed by microscope. Mitotic metaphase chromosomes were microphotographed and were karyotypically analyzed. Chromosomes were classified into several types, following the nomenclature of Levan et al. (1964). Voucher specimens are deposited in the Herbarium of Okayama University of

Science (OKAY).

### Results and Discussion

After examining chromosomes of *Aucuba japonica* collected from 16 localities in the Chugoku and Shikoku Districts, all individuals proved to be diploid with  $2n = 16$  (Table 1). Figures 1 and 2 show somatic chromosomes and karyotypes of diploid races of *A. japonica*, respectively. The length of somatic chromosomes are from 3.6 to 7.1  $\mu\text{m}$ , and karyomorphologically, the complement is mono-modal. Eight chromosome pairs were recognized by configuration of chromosomes: three large-sized pairs with subterminal to submedian chromosomes, one medium-sized pair with median chromosomes, and four small-sized pairs with subterminal to median chromosomes. The smallest pair had comparatively large satellites on the short arms. Kurosawa (1971) observed several diploid individuals of *A. japonica* collected from the Kyushu District and the Okinawa Island. Although the present study has confirmed that the smallest pair has satellites, it is not clear whether the large-sized pairs have satellites or not. Other methods, such as FISH, C-banding, or silver-staining methods, should be applied to confirm the number and position of satellites.

During the analysis of karyomorphology, we found that 24 individuals out of the 59 individuals possessed a heterogeneous karyotype with one median and one submedian chromosomes in the fourth longest pair ("heterotype"). The remaining 35 individuals possessed median chromosomes in the pair ("homotype") (Figs. 1, 2). All the "heterotypes" were found to be male individuals, and "homotypes" to be female individuals (Table 1). As "heterotype" individuals were distributed throughout the research area and found in four populations with "homotype" individuals (Table 1), we have concluded that the karyomorphological variation in diploid races of *A. japonica* is not geographi-

Table 1. Collection data, chromosome numbers, and karyotypes of diploid races of *Aucuba japonica*

Locality (collector, voucher specimen number, altitude)	Sex	Number of individuals examined	Chromosome number (2n)	Karyotype
<b>Okayama Prefecture</b>				
Ukankei, Shimokamo, Kibichuo-cho (M. Tsusaka & al. TS04100602, 150 m)	♂	1	16	hetero*
Ukankei, Shimokamo, Kibichuo-cho (M. Tsusaka & al. TS04100603, 04100610, 150 m)	♀	2	16	homo**
Ukankei, Shimokamo, Kibichuo-cho (M. Tsusaka 0505032XF, 150 m)	♀	4	16	homo
Ukankei, Shimokamo, Kibichuo-cho (M. Tsusaka 0505032XM, 150 m)	♂	2	16	hetero
Iwayakei, Kawakami-cho, Takahashi-shi (M. Tsusaka 03052401, 150 m)	♀	1	16	homo
Nunose, Bitchu-cho, Takahashi-shi (M. Tsusaka & al. 050220A, 150 m)	♂	2	16	hetero
<b>Shimane Prefecture</b>				
Fukamachi pond, Nishikawazu-cho, Matsue-shi (S.-J. Lin 04041901, 30 m)	♂	1	16	hetero
Naku, Okinoshima-cho (M. Tsusaka 03102502, 320 m)	♀	1	16	homo
<b>Yamaguchi Prefecture</b>				
Kamitama, Tamagawa-cho (M. Tsusaka 03041303, 10 m)	♀	1	16	homo
<b>Tokushima Prefecture</b>				
Minamikumaso, Kamiyama-cho (M. Tsusaka 05072201, 90 m)	♀	1	16	homo
Minamikumaso, Kamiyama-cho (M. Tsusaka 05072201, 90 m)	♂	1	16	hetero
<b>Kochi Prefecture</b>				
Higashi-ishihara, Tosa-cho (M. Tsusaka 050314A2, 560 m)	♀	1	16	homo
Higashi-ishihara, Tosa-cho (M. Tsusaka 050314A2, 560 m)	♂	1	16	hetero
Shiraiwado, Kagami, Kochi-shi (M. Tsusaka 050314, 285 m)	♂	1	16	hetero
Sakagawa, Tosayamada-cho (M. Tsusaka 050313A1, 82 m)	♀	9	16	homo
Sakagawa, Tosayamada-cho (M. Tsusaka 050313A1, 82 m)	♂	11	16	hetero
Nagasawa, Ino-cho (M. Tsusaka 050314A1, 610 m)	♀	8	16	homo
Nagasawa, Ino-cho (M. Tsusaka 050314A1, 610 m)	♂	4	16	hetero
Kususe, Ino-cho (M. Tsusaka TS03060604, 40 m)	♀	1	16	homo
Iibo, Yusu-hara-cho (M. Tsusaka 04101301, 490 m)	♀	2	16	homo
Shimokurechi, Kubokawa-cho (M. Tsusaka 04101303, 350 m)	♀	1	16	homo
Ooino, Kubokawa-cho (M. Tsusaka 04101302, 320 m)	♀	1	16	homo
<b>Ehime Prefecture</b>				
Ohkuki, Uchiko-cho (M. Tsusaka 04101202, 160 m)	♀	2	16	homo

\*hetero: the fourth-longest pair with heterogeneous chromosomes.

\*\*homo: the fourth-longest pair with homogeneous chromosomes.

cal nor populational variation, but corresponds to the sexuality. Karyomorphological variation corresponding to sexuality in *A. japonica* is reported for the first time. It is

therefore supposed that the fourth chromosome pairs are sex chromosomes.

The reports of sex chromosomes in dioecious plants have been mainly for herbs

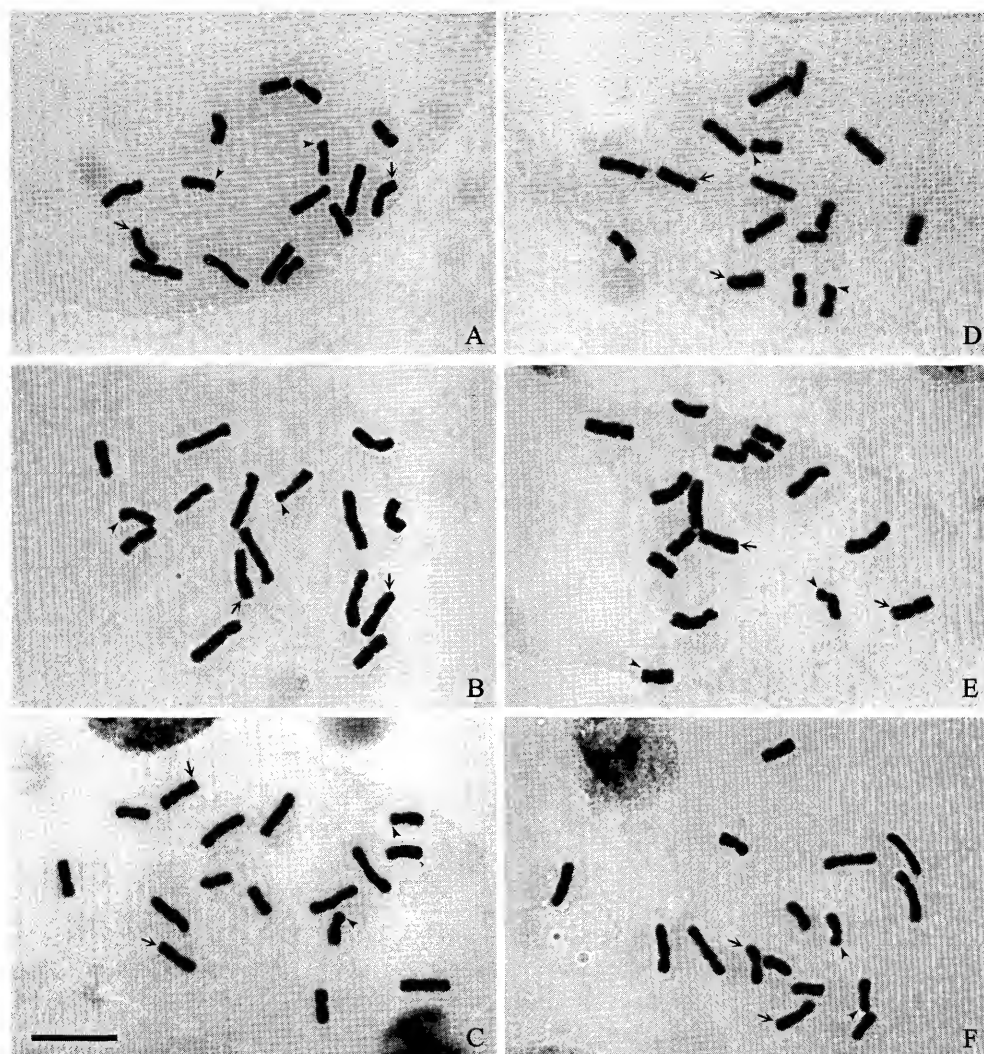


Fig. 1. Somatic chromosomes of diploid races of *Aucuba japonica*. A–C: Female, D–F: Male. A: Ukankei, Okayama Pref. B: Nagasawa, Kochi Pref. C: Iibo, Kochi Pref. D: Nishikawazu-cho, Shimane Pref. E: Ukankei, Okayama Pref. F: Takahashi-shi, Okayama Pref. Arrows indicate the fourth-longest pair with homogeneous chromosomes (A–C) and heterogeneous chromosomes (D–F). Arrowheads indicate satellite chromosomes. Bar = 10  $\mu$ m.

(Blackburn and Harrison 1924, Meurman 1925, Lindsay 1930, Löve 1943, Sinoto 1929, Winge 1923), and relatively few for woody plants (Blackburn and Harrison 1924, Meurman 1925, Lindsay 1930, Sinoto 1929). There is no report on sex chromosomes for

dioecious evergreen shrubs like *A. japonica*. The heteromorphic chromosomes observed in diploid races of *A. japonica* are supposed to be sex chromosomes and this is the first report of sex chromosomes for evergreen shrub.

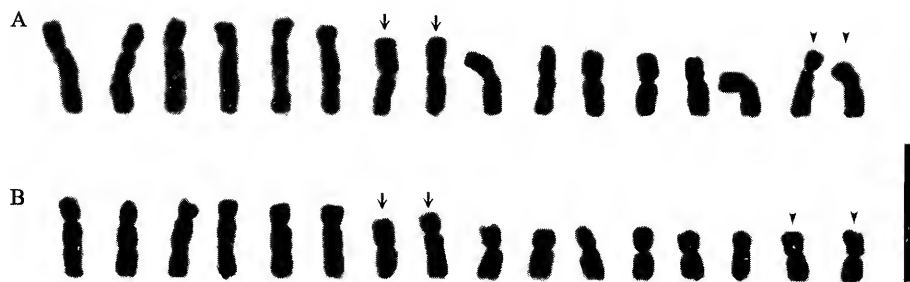


Fig. 2. Karyotypes of diploid races of *Aucuba japonica*. A: Nagasawa, Kochi Pref. (♀). B: Nishikawazu, Shimane Pref. (♂). Arrows indicate the fourth-longest pair with homogeneous chromosomes (A) and heterogeneous chromosomes (B). Arrowheads indicate satellite chromosomes. Bar = 10  $\mu$ m.

We thank Mr. Shungo Kariyama, Kurashiki Museum of Natural History, Mr. Makoto Ogawa, Tokushima Prefectural Museum, and Dr. Tetsuo Ohi, Botanical Garden of the University of Tokyo, for their valuable information on distribution of *Aucuba japonica*. We also appreciate Dr. Su-Jang Lin, Shimane University, for sending a material. This study is partly supported by the Yakumo Foundation for Environmental Science in 2005 (to M. T.).

#### References

- Blackburn K. B. 1923. Sex chromosomes in plants. *Nature* **112**: 687–688.
- and Harrison J. W. H. 1924. A preliminary account of the chromosomes and chromosome behaviour in Salicaceae. *Ann. Bot.* **38**: 361–378.
- Funabiki K. 1958. Distribution and polyploidy of angiosperms I. *Kromosomo* **37–38**: 1253–1267.
- Hara H. 1966. Cornaceae. *The Flora of Eastern Himalaya*. p. 744. University of Tokyo Press, Tokyo.
- 1989. Cornaceae. In: Satake Y., Hara H., Watari S. and Tominari T. (eds.), *Wild Flowers of Japan*, *Woody Plants II*: 109–112. Heibonsha, Tokyo (in Japanese).
- Kihara H. and Ono T. 1923. Cytological studies of *Rumex* L. *Bot. Mag. Tokyo* **37**: 84–90.
- and Yamamoto Y. 1935. Chromosomenverhältnisse bei *Aucuba chinensis* Benth. *Agric. Hortic. Tokyo* **10**: 2485–2495.
- Kurosawa S. 1971. Cytotaxonomical studies on the genus *Aucuba*. *J. Jpn. Bot.* **46**: 231–238.
- Levan A., Fredga K. and Sandberg A. A. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* **52**: 201–220.
- Löve A. 1943. Cytogenetic studies on *Rumex* subgenus *Acetosella*. *Hereditas* **30**: 1–136.
- Lindsay R. H. 1930. The chromosomes of some dioecious angiosperms. *Amer. J. Bot.* **17**: 152–174.
- Meurman O. 1925. The chromosome behaviour of some dioecious plants and their relatives with special reference to the sex chromosomes. *Soc. Sci. Fennica, Commentat Biol.* **2**: 1–105.
- 1929. Chromosome numbers in the family Cornaceae. *Mem. Soc. Fauna Flora Fennica* **6**: 95–100.
- Noshiro S. 1999. Cornaceae. In: Iwatsuki K., Boufford D. E. and Ohba H. (eds.), *Flora of Japan II*: 254–258. Kodansha, Tokyo.
- Ohi T., Kajita T. and Murata J. 2003. Distinct geographic structure as evidenced by chloroplast DNA haplotypes and ploidy level in Japanese *Aucuba* (Aucubaceae). *Amer. J. Bot.* **90**: 1645–1652.
- Sinoto Y. 1929. Chromosome studies in some dioecious plants, with special reference to the allosomes. *Cytologia* **1**: 109–191.
- Sugiura T. 1927. Some observations on the meiosis of the pollen mother cells of *Carica papaya*, *Myrica rubra*, *Aucuba japonica* and *Beta vulgaris*. *Bot. Mag. Tokyo* **41**: 219–224.
- Viinikka Y. 1970. A comparative study of mitotic and meiotic chromosomes of *Aucuba japonica* Thunb. *Ann. Bot. Fennici* **7**: 203–211.

Winge Ø. 1923. On sex chromosomes, sex determination and preponderance of females in some

dioecious plants. Compt. Rend. Trav. Lab. Carlsberg 15: 1-26.

津坂真智子, 池田 博, 星野卓二: アオキ (ミズキ科) の2倍体における核形態学的解析

ミズキ科アオキ (*Aucuba japonica* Thunb.) の二倍体 ( $2n=16$ ) について, 雌雄性を考慮して核型分析を行った. 中国・四国地方産のアオキ16地点59個体について解析を行ったところ, すべての個体で最小の染色体対に付随体がみられ, これまでの報告と一致した. しかし, これまで指摘されていた最大の染色体対と三番目に長い染色体対にあるとされる二次狭窄については, 今回の研究では明瞭に識別することはできなかった. また, 四番目に長い染色体対に中部動原体染色体を2本もつ

ホモ型と, 中部動原体染色体と次中部動原体染色体を一本ずつもつヘテロ型の組み合わせをもつ個体が見出された. この変異は地理的なものではなく, ホモ型の組み合わせをもつ個体はすべて雌であり, ヘテロ型の組み合わせをもつ個体はすべて雄であることが明らかになった. したがって, アオキの二倍体は, 性によって異なる核型を示し, 性染色体をもっていると考えられる.

(岡山理科大学総合情報研究科  
数理・環境システム専攻)